

Nutritional and microbial analysis of bully sticks and survey of opinions about pet treats

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Abstract — The objectives of this study were to measure the caloric density of bully sticks, to analyze the bully sticks for bacterial contamination, and to assess owner opinions about these and other pet treat products. Mean caloric density was 15 kcal/inch (38 kcal/cm) [range: 9 to 22 kcal/inch (23 to 56 kcal/cm), 2.96 to 3.07 kcal/g]. Of 26 bully sticks that were tested for bacterial contamination 1 (4%) was contaminated with *Clostridium difficile*, 1 was contaminated with methicillin-resistant *Staphylococcus aureus* (MRSA), and 1 with a tetracycline resistant *Escherichia coli*.

Résumé — Analyse nutritionnelle et microbienne des bâtonnets en peau de buffle et sondage d'opinion à propos des gâteries pour animaux de compagnie. Les objectifs de cette étude consistaient à mesurer la densité calorique des bâtonnets en peau de bovin, à analyser les bâtonnets en peau de bovin pour une contamination bactérienne et à évaluer les opinions des propriétaires à propos de ces gâteries et d'autres gâteries pour animaux de compagnie. La densité calorique moyenne était de 15 kcal/pouce (38 kcal/cm) [écart : de 9 à 22 kcal/pouce (de 23 à 56 kcal/cm), de 2,96 à 3,07 kcal/g]. Parmi les 26 bâtonnets en peau de bovin qui ont été testés pour une contamination bactérienne, 1 (4 %) était contaminé par *Clostridium difficile*, 1 était contaminé par *Staphylococcus aureus* résistant à la méthicilline (SARM) et 1 par *Escherichia coli* résistant à la tétracycline.

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Introduction

Pet treats are a fast-growing segment of the pet food industry. There are many types of pet treats, including hard biscuits, soft treats, and edible manufactured bones. There is a wide variety of treats derived from parts of animals other than skeletal muscle. These include familiar products that have been on the market for many years, such as cattle hooves or skin (i.e., rawhide chews) and pig ears, but now range from lungs to tracheas to hearts. One treat for dogs is the bull or steer penis, commonly known as “bully” or “pizzle” sticks.

There are a number of potential concerns with bully sticks and other treats. One is as an additional source of calories.

Obesity is a common problem in dogs and, in the authors' clinical experience, most owners do not consider treats to contain a significant number of calories. Therefore, owners may be unknowingly providing additional calories to their dogs by feeding bully sticks.

Another possible concern for bully sticks and other similar treats is bacterial contamination. Outbreaks of human salmonellosis have been associated with contact with contaminated pig ears (contamination rates between 41% to 51%) (1–3). A more recent study showed that the prevalence of *Salmonella* contamination had decreased substantially to 4% but resistance remained a problem with isolates having resistance to 7 antimicrobials (4). Similar studies on bacterial contamination and resistance patterns have not been published for bully sticks. Furthermore, recent identification of emerging issues of community-associated *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus* (MRSA) infection in humans (5–7) and identification of these pathogens in retail food products (8–12) raise the question of whether pet treats might be sources of exposure.

Finally, in the authors' clinical experience, many pet owners and even veterinarians appear to be unaware of what pet treats are made of, particularly in the case of bully sticks. Understanding potential risks and owner perceptions about treats may enhance communication with dog owners. Therefore, the purpose of this study was to measure the caloric density of bully sticks and to analyze these products for bacterial contamination. In addition, a survey was performed to assess owner opinions about pet treats, including bully sticks.

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Materials and methods

A convenience sample of 26 bully stick treats was purchased from retail outlets in the United States ($n = 16$) and Canada ($n = 10$). Although some of the treats were produced by the same manufacturers, all were different products (i.e., 26 different brands). A sample of each available bully stick product was purchased for each retail outlet visited to avoid selection bias.

Five 5- to 7-inch (13- to 18-cm) bully sticks purchased in the United States were randomly selected and submitted for proximate analysis at a commercial laboratory (Eurofins Scientific, Des Moines, Iowa, USA). Total length, diameter, and weight of each stick were recorded, and crude protein, crude fat, crude fiber, moisture, and ash were analyzed. Non-fiber carbohydrate was calculated (100-moisture-crude protein-crude fat-crude fiber-ash), and the kilocalories (kcal) per gram were calculated using modified Atwater factors on an as-fed basis (13). Using kcal/g, weight, and length, the kcal/treat and kcal/inch (kcal/cm) were calculated.

Microbiological testing was performed on all 26 treats. Treats were tested for the primary organism of interest (*Salmonella* spp.), but also for *Clostridium difficile*, MRSA, and generic *Escherichia coli*. Antimicrobial susceptibility testing of *E. coli* isolates was also performed.

A sample of approximately 10 g was inoculated into approximately 30 mL of *C. difficile* moxalactam norfloxacin (CDMN) broth (Oxoid, Nepean, Ontario) with 0.1% sodium taurocholate and incubated anaerobically at 37°C for 7 d. An aliquot of the broth was alcohol shocked with an equal volume of anhydrous ethanol for 1 h. This mixture was then centrifuged for 10 min at $3980 \times g$. The supernatant was discarded and the pellet was streaked onto a CDMN agar plate and incubated anaerobically at 37°C for 48 h. Suspicious colonies were subcultured onto blood agar and confirmed as *C. difficile* by Gram stain appearance, colony morphology, characteristic odor, and production of L-proline aminopeptidase.

Another sample of approximately 10 g was inoculated into 30 mL of enrichment broth consisting of 10 g tryptone/L, 75 g sodium chloride/L, 10 g mannitol/L, and 2.5 g of yeast extract/L. After 24 h incubation at 35°C, 5 to 10 μ L of broth were inoculated onto MRSA chromogenic agar (Becton Dickinson, Franklin Lakes, New Jersey, USA). Plates were incubated at 35°C and read after 24 h and 48 h. Isolates were identified as *S. aureus* by colony morphology, Gram stain appearance, catalase reaction, coagulase reaction, and *S. aureus* latex agglutination test (Bio-Rad Laboratories, Mississauga, Ontario). Methicillin-resistance was confirmed by penicillin binding protein 2a latex agglutination test (Oxoid).

Salmonella and *E. coli* testing was performed following pre-enrichment of treats in buffered peptone water (BPW) at 37°C for 24 h. For *Salmonella*, 0.1 mL of the BPW mixture was inoculated into modified semi-solid Rappaport-Vassiliadis agar (Oxoid) and incubated at 42°C for 24–72 h. Presumptive colonies were plated on MacConkey agar (Becton Dickinson) and xylose lysine tergitol 4 agar (Oxoid) and incubated at 37°C for 24 h, and non-lactose fermenting colonies were inoculated on tryptic soy agar (Becton Dickinson). Biochemical testing

was conducted using Christensen's urea, triple sugar iron, and agglutination in *Salmonella* O antiserum Poly A-I & Vi (all from Becton Dickinson).

For *E. coli*, 50 mL of the BPW mixture was combined with 50 mL of double strength *E. coli* broth (Becton Dickinson) and incubated at 37°C for 18 to 24 h. A loopful of rinse was plated on Eosin Methylene Blue agar (Becton Dickinson) and incubated at 37°C for 18 to 24 h. Presumptive *E. coli* colonies were transferred to MacConkey agar for purification and incubated at 37°C for 18 to 24 h. Isolated *E. coli* colonies were transferred onto tryptic soy agar plates and incubated at 37°C for 18 to 24 h. Confirmation testing of *E. coli* was conducted using Kovac's indole spot reagent (Remel, Ottawa, Ontario) and Simmon's citrate agar (Becton Dickinson). Antimicrobial susceptibility testing for *E. coli* isolates was conducted using an automated broth microdilution system (Sensititre, Trek Diagnostic Systems, East Grinstead, West Sussex, United Kingdom). The National Antimicrobial Resistance Monitoring System (NARMS) susceptibility panel CMV1AGNF was used with methods described by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (14,15). The breakpoints for resistance are those used by CIPARS and NARMS, which were derived from the Clinical and Laboratory Standards Institute (CLSI) where available (14,15). A lower breakpoint for ceftriaxone was used in this study, and is adapted from the CLSI Informational supplement M100-S20 (16).

For molecular typing, *C. difficile* isolates were typed by PCR ribotyping, as described elsewhere (17). When the ribotype was known to be a recognized international ribotype through previous typing of reference strains from the Public Health Laboratory Service Anaerobic Reference Unit, Cardiff, UK, the appropriate numerical designation (i.e., 078) was used. Otherwise, internal nomenclature was used. Genes encoding production of toxins A (*tcdA*) and B (*tcdB*) were evaluated using polymerase chain reaction (PCR) (18,19). Detection of CDT (binary toxin) was performed using PCR directed at *cdtB*, the binding component (20).

The MRSA isolates were typed by sequencing of the X region of the protein A gene (spa typing) (21) and classified using the Ridom system (22). Real-time PCR was used to detect the *lukF* and *lukS* Panton-Valentine leukocidin genes (23). Positive and negative controls were performed with each PCR run.

A 20-question Web-based survey was developed with the assistance of the Tufts University Office for Institutional Research and contained questions regarding the participant's pet ownership, opinions regarding dog foods and treats, and participants' background (survey available from the corresponding author upon request). Respondents were also asked to indicate if they were a veterinarian, veterinary technician, or dog breeder; this category is referred to as "professional category" hereafter. The study was reviewed and approved by Tufts University's Institutional Review Board. The survey was posted online for public participation for 60 d. A survey was considered to be complete if $\geq 80\%$ of the questions were answered. No incentive was offered for participation in the survey, and all responses were anonymous.

Data were examined graphically. Data are presented as mean \pm standard deviation (SD) (normally distributed data) or median and range (skewed data). Results were compared by subcategories using Chi-squared analysis. Percent comparisons were calculated from the total survey respondents answering the individual question, unless otherwise stated. All analyses were performed using commercial statistical software (Systat 12.0; SPSS, Chicago, Illinois, USA).

Results

Mean caloric density of the 5 bully sticks was 3.01 kcal/g (range: 2.96 to 3.07 kcal/g). Based on the variable length (mean = 5.71 ± 0.71 inches or 14.49 ± 1.80 cm) and diameter (mean = 2.17 ± 0.34 inches or 5.50 ± 0.86 cm), this resulted in a mean total caloric density of 88 kcal/treat (range: 45 to 133 kcal/treat) or 15 kcal/inch (range: 9 to 22 kcal/inch).

One of 26 samples (4%) was positive for *Clostridium difficile*. The isolate was a toxigenic strain with a ribotype pattern that has not been previously identified in the authors' collection of isolates from animals, food, and humans. One other sample (4%) was positive for MRSA. The isolate was spa type t011, a sequence type 398 (ST398) strain. Generic *E. coli* were isolated from 7 of 26 samples (27%). Of these 7 isolates, 1 was resistant to tetracycline and the other 6 were pan-susceptible to the antibiotics tested.

The survey was completed by 852 adults from 44 US states and 6 countries. Most respondents (791; 92.8%) were dog owners and female (738; 86.6%). Professional categories included veterinarians ($n = 81$), veterinary technicians ($n = 66$), and dog breeders ($n = 112$). Most respondents ($n = 483$; 57.2%) answered that ingredients were the most important factor when choosing a dog food. Other answers included recommendation from a veterinarian ($n = 205$; 24.3%), recommendation from a breeder ($n = 60$; 7.1%), the label says it is most appropriate for the individual dog/breed ($n = 29$; 3.4%), convenience ($n = 29$; 3.4%), price ($n = 28$; 3.3%), and recommendation from a pet store ($n = 10$; 1.2%). Respondents' primary source of information for nutritional advice was the veterinarian ($n = 381$; 45.2%), the internet ($n = 133$; 15.8%), breeder/trainer ($n = 95$; 11.3%), books/magazines ($n = 58$; 6.9%), veterinary clinic staff ($n = 47$; 5.6%), friends/family ($n = 42$; 5.0%), pet store staff ($n = 18$; 2.1%), and other ($n = 68$; 8.1%). A lower proportion of breeders (17%) reported that their primary source of information was the veterinarian compared with any of other professional categories (general respondents: 43%; veterinarian technicians: 68%; veterinarians: 80%; $P < 0.001$).

For respondents who were dog owners ($n = 791$), the survey asked the type of food that composed the largest proportion of the diet and any foods that were included in the dogs' diets. Most respondents fed dry food as the major component of the diet (663; 83.8%), but 85 (10.8%) fed either a commercial or homemade raw meat diet. Breeders were significantly more likely to feed a raw diet (either commercial or homemade; 23%) or a homemade diet (raw or cooked; 15%) compared with any of the other professional categories ($P < 0.001$). When asked about ingredients that they avoided in pet food, 454 (57.4%) avoided by-products, while 450 (56.9%) avoided preservatives

Table 1. Answers from 752 respondents to the question, "Which of the following is an accurate description of bully sticks?" (total number with percentage in parentheses)

Bull penis	418 (55.6%)
Cow tendon	154 (20.5%)
Cow muscle	47 (6.3%)
Rolled up sheep skin	8 (1.1%)
Didn't know	115 (15.3%)
Other	10 (1.3%)

and 295 (37.3%) avoided grains. Sixty-nine respondents (8.7%) listed other ingredients that they avoided which included artificial colors, beet pulp, chicken, beef, ingredients from China, soy, lamb, genetically modified organisms, garlic, wheat gluten, sugar, dairy, carbohydrates, cheese, chemicals, citric acid, and rosemary.

When asked what was contained in pet food by-products, most of the 773 respondents who answered this question ($n = 674$; 87.2%) answered correctly that internal organs were included (13). However, many also responded that ingredients that are specifically prohibited from by-products [Association of American Feed Control Officials (AAFCO) Feed Ingredient Definition 9.3 (13)] were included, such as hooves (466; 60.3%), horns (366; 47.4%), feces (167; 21.6%), road kill (103; 13.3%), and euthanized pets (99; 12.8%). Veterinarians and veterinary technicians were less likely than other professional categories (breeders and general respondents) to incorrectly respond that by-products contain these other items.

Two hundred forty-three dog owners (30.7%) fed rawhide chews to their dogs and 180 (22.8%) fed bully sticks. Of the respondents who fed bully sticks, 71% also stated that they avoided by-products. Four hundred eighteen of the 752 respondents for this question (55.6%) correctly identified that bully sticks were derived from bull penis but a variety of other responses also were provided (Table 1). Of the respondents who fed bully sticks, 28% did not correctly identify the source of bully sticks. While a higher proportion of veterinarians (62%) correctly identified the source of bully sticks compared to general respondents (44%; $P = 0.006$), 38% of veterinarians had incorrect responses to this question.

Calories in a 12" bully stick were underestimated by 50% of respondents. Fifty percent correctly answered 150 kcal but 38% answered 70 kcal, 9% answered 20 kcal, and 3% answered 0 kcal. Veterinarians had a higher rate of correct responses (62%) compared with all other professional categories. Potential risks of bully sticks were also questioned in the survey. The most frequent response from the 812 respondents on this question was that they can get stuck in the stomach or intestine ($n = 697$; 85.8%). Response rate to other potential risks included: they can be contaminated with bacteria ($n = 477$; 58.7%), they can break a dog's teeth ($n = 242$; 29.8%), they can contain antibiotics ($n = 106$; 13.1%), and they have no risks ($n = 34$; 4.2%).

Discussion

The number of calories measured in the 5 bully stick samples was similar on a weight basis. However, the length and width of bully sticks varies widely so total calories in an individual treat will vary accordingly. Nonetheless, the results show that bully sticks could provide between 54 to 132 kcal for a 6" bully stick

and 108 to 264 for a 12" bully stick. These calories may not be accounted for by the dog owner (50% of respondents in the current study underestimated the number of calories in bully sticks), especially if bully sticks are fed frequently. If the mean for a 6" bully stick were used (i.e., 90 kcal), 1 bully stick daily would be equivalent to 9% of the daily calorie requirements for a 50-pound (23-kg) dog and 30% of the daily calorie requirements for a 10-pound (4.5-kg) dog (24). With the high rate of obesity in dogs, veterinarians should be aware of bully sticks and other pet treats as a source of calories in a dog's diet and should consider not only the dog food, but also pet treats and table food. Calorie information is currently not required on pet treats or on most pet foods so is not readily accessible for veterinarians and pet owners. The calorie information from the current study provides some information on calories in bully sticks.

The contamination rate in the current study [1 each (4%) contaminated with *Clostridium difficile*, MRSA, or tetracycline resistant *E. coli*] was relatively low but should be studied further to understand potential risks to pets and to human members of the household. *Salmonella* spp. were not isolated from any of the bully sticks in the current study but the low sample size may have limited the ability to detect a low rate of contamination. The human health relevance of contamination with *C. difficile* and MRSA is unknown. The *C. difficile* strain identified here has not been found in the authors' collection of over 2000 human isolates; however, this does not exclude the possibility that it can cause disease. The MRSA strain that was identified is a livestock associated strain that is common in pigs internationally and is of concern in humans in some regions. Since human infection from handling *Salmonella*-contaminated treats can occur, it is plausible that bully sticks could be a source of infection. Additionally, bully sticks could be vehicles for MRSA colonization or extra-intestinal infection. Additional information is needed on the roles of processing, packaging, and cross-contamination in the safety of pet treats. Pet owners should be aware that these types of pet treats can be contaminated with bacteria and should follow standard recommendations when handling treats, particularly handwashing after contact with treats and ensuring that high risk individuals (very young, elderly, pregnant, immunocompromised) avoid all contact with raw animal-product based treats.

It appears from the survey results that many people have misconceptions about bully sticks, although bully sticks were fed to the dogs of 23% of respondents. Seventy-one percent of people feeding bully sticks also stated that they avoided by-products in pet foods and 28% did not correctly identify what bully sticks were made of. Manufacturers of bully sticks are not required to state that bully sticks are derived from bull/steer penis, and often list the ingredients as "bull pizzle" or even misleadingly as "cow muscle." While veterinarians had a higher rate of correct responses for the source of bully sticks, 38% of veterinarians incorrectly identified them. This suggests that both veterinarians and pet owners would benefit from increased awareness about the source of bully sticks so that they can make informed purchasing and feeding decisions.

Veterinarians were the most commonly reported primary source of information about nutrition so this offers an important

opportunity to provide objective and accurate information. For example, a large proportion of respondents (57%) stated that they avoided by-products in pet foods, but most incorrectly identified ingredients that comprise by-products. Providing accurate information on nutrition and pet foods can assist owners in making more informed decisions about their pet's food. While veterinarians or veterinary clinic staff were the primary source of information for many respondents, the primary resources for other respondents (e.g., the internet) may provide less reliable information on nutrition and pet foods.

There are a number of limitations to the current study. Studies with a larger sample size are warranted to determine whether the calorie content and contamination rate found in this small study is representative of all bully sticks (or even other types of pet treats). While using an online survey allowed for a relatively large pool of respondents, respondents were primarily recruited through e-mail and other electronic techniques, limiting the respondents to individuals with computer and internet access. Also, there may have been bias in the respondents, as those who chose to respond to the survey may have had a stronger opinion about dog foods and treats. Similarly, a larger proportion of respondents were dog owners, which may have contributed to bias. In addition, there was no way to prevent individuals taking the survey from checking answers to the factual questions (e.g., what are bully sticks made from?) so it is not possible to know whether these questions accurately assessed the respondents' knowledge before taking the survey. These limitations may reduce the relevance of the results for other populations. Veterinarians and pet owners, however, should be aware of the high calorie content and potential for bacterial contamination of bully sticks.

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Book Review

Compte rendu de livre

Self-Assessment Color Review: Feline Infectious Diseases

Hartmann K, Levy JK, eds. 2011. Manson Publishing/The Veterinary Press: London, UK. 224 pp. ISBN: 9781-8407-6099-6, UK £24.95 (pbk).

The Self-Assessment Color Review series is comprised of books in various areas of veterinary medicine that use a case-based approach. This volume presents an overview of feline infectious diseases written as 199 short illustrated cases. The description of each case is given in a paragraph or two and is followed by a few questions. Cases are illustrated with one or two images that may show clinical lesions, radiographs, photomicrographs, histology sections, etc. The detailed answer for each set of questions is found by turning the page, making it convenient to read through the cases and test one's knowledge. For example, a case of *Otodectes cyotis* infection is presented with a signalment and history as well as photos of the skin lesions and the mites. The reader is then asked questions such as how the infection is transmitted and what treatment options are available. The cases

include a variety of feline infectious diseases — viral, bacterial, fungal, and parasitic. The editors and their 13 international contributors use a practice-oriented approach to presenting the cases, closely approximating the way such diseases would appear in clinical practice, thus reinforcing clinical skills. The front of the book shows the cases classified by organ system or type of infection, making it easy to find cases in particular subject areas. Finally, the book also contains a table of normal reference ranges for physical examination findings, complete blood count, coagulation and biochemistry panels, and urinalysis.

This book would be most useful to veterinary students in clinical rotations, but also valuable for clinicians who wish to review and improve their knowledge of feline infectious diseases. The book would also be useful for clinicians studying for further qualifications. The short case format and accessible style make it easy to pick up the book, browse and view cases, and learn a few new facts in the space of a few minutes free time.

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